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PRINCIPAL INVESTIGATOR: Paul C. Wang, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Washington, DC 20059

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Nuclear magnetic resonance (NMR) imaging and spectroscopy have emerged as one of the most promising techniques to improve the specificity of the diagnosis and staging of breast cancer. They have been widely used in studying of the tumor energy metabolism, vascular and oxygenation and response to treatment of breast cancer. NMR techniques intrinsically have weak signals, which limit the ultimate resolution and sensitivity. We propose to use high temperature superconductor (HTS) to construct NMR probe to reduce the probe noise and significantly improve the detection sensitivity of the technique. The probe will be constructed with YBCO material and to be tested on two well defined experiments: an in vivo cell metabolism study on a 9.4 T spectrometer and an in vivo tumor bearing animal study on a 4.7 T scanner. During the first year of the study, several tasks are completed. There are: (1) construction of and testing a cell perfusion apparatus (2) start to grow MCF7 cells (3) designing and construction of a HTS probe and a room temperature probe (4) to upgrade the software on the 200 MHz NMR machine (5) implementing the diffusion imaging technique (6) to relocate the 400 MHz machine and renovation of the laboratory.

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Diane C Wang 10/16/97

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I. INTRODUCTION

Conventional mammography has been shown to play an important role in detection and staging of breast cancer in older women. Younger women who frequently have radiodense breast tissue or women with silicon implants, rendering breast cancer diagnosis with conventional mammography is problematic. When mammographic findings and clinical findings concur regarding the possibility of a lesion being malignant, usually a fine-needle aspiration biopsy will be performed for definitive diagnosis. The false positive rate is high; only 20-30% of lesions suspicious for cancer at mammogram are actually positive for cancer at biopsy. In general, mammography is limited to detect a tumor several millimeters or larger in size. Because of difficulty with early detection, clinicians are sometimes limited to treat larger size cancers, which in many cases have already metastasized. Accurate definition of tumor size, number, and margins is highly critical in the clinical determination of conservation treatment versus mastectomy. A role exists for an imaging method that can improve sensitivity for detection of small lesion and to improve the specificity for better staging of the disease. To provide the best chance of overall survival, breast cancers need to be accurately staged for systemic treatment and optimal conservation surgery. Traditionally, the gold standards for such assessments are clinicopathological staging and histopathological typing and grading of malignancy. In the classical histopathological approach problems exist inherently, predominantly, the accuracy of the initial biopsy procedure and the variable skills applied to its histological assessment. Development of a new modality to remove sampling errors, improve specificity and produce a grading of tissues that relates to established biological criteria would be very useful. Over the last few years, magnetic resonance imaging (MRI) and spectroscopy (MRS) have emerged as one of the most promising clinical tools to fill the gap between clinical needs and information obtained by conventional breast imaging and pathological methods. Preliminary results indicate that MRI may be more sensitive than conventional x-ray mammography in detecting small lesions. Cancers have typical metabolic characteristics in ^{31}P and ^{1}H MRS including high levels of phospholipid metabolites and a cellular pH more alkaline than normal. Although these alone are not unique for cancer but they are very useful diagnostic information in appropriate clinical settings. MRS is capable of distinguishing benign and malignant lesions in a particular anatomical site and to be a specific diagnostic discriminant in a particular situation. It has been demonstrated to be useful to improve the specificity of the MR imaging of breast. Some metabolic characteristics appear to be prognostic indices and correlate well with the response of treatment. The improvement of specificity will reduce the number of biopsies performed to confirm false-positive mammographic findings and more effectively to assess the results of treatment. Many of these progresses are based on the advances of NMR studies of the perfused breast cancer cells and tumor-bearing animal models. One of the major limitations of the application of NMR methods both in vitro and in vivo is its low sensitivity. The sensitivity determines the ultimate cancer detection capability and the resolution in image and in spectrum. In this study, a high temperature superconductor working at very low temperature will be used to reduce electronic noise and significantly improve the sensitivity of detection. It will dramatically increase the sensitivity and improve the resolutions. Clinically, the HTS probe will significantly improve the sensitivity of detection and specificity of MRI and MRS techniques. It will provide a more accurate diagnosis and it may become possible for early prediction of tumor response to therapy.

II. ASSUMPTIONS

Over the last few years, magnetic resonance imaging (MRI) and spectroscopy (MRS) have emerged as one of the most promising clinical tools to fill the gap between clinical needs and information obtained by conventional breast imaging and pathological methods (1-9). Preliminary results indicate that MRI may be more sensitive than conventional x-ray mammography in detecting small lesions. The overall advancement of MR imaging of breasts can be summarized as: (a) earlier detection of breast cancer, particularly in the radiographically opaque breast; (b) more depiction of carcinoma; (c) detection of multifocal disease better than mammography; (d) better delineation of tumor margin; (e) assisting treatment decision making; (f) better definition of leakage or rupture of implants; (g) documentation of therapeutic responses and reoccurrence of the disease after surgery. However, without dynamic contrast agents, study of the appearances of some benign and malignant lesions overlapped. The sensitivity of MRI to detect lesions in the breast is generally high, better than 90%. However, the specificity of the disease type especially its value of discerning benign and malignant solid tumors, varies from 39% to 89% in different studies (10-12). MRS permits non-invasive examination of metabolic characteristics of human cancers in a clinical environment (13-21). Many nuclei are accessible in MRS technique including ^{1}H, ^{13}C, ^{19}F, ^{23}Na and ^{31}P. Cancers have typical metabolic characteristics in ^{31}P and ^{1}H MRS including high levels of phospholipid metabolites and a cellular pH more alkaline than normal. Although these alone are not unique for cancer but they are very useful diagnostic information in appropriate clinical settings. MRS is capable of distinguishing benign and malignant lesions in a particular anatomical site and to be a specific diagnostic discriminant in a particular situation. It has been demonstrated to be useful to improve the specificity of the MR imaging of breast. Some metabolic characteristics appear to be prognostic indices and correlate well with the response of treatment. The improvement of specificity will reduce the number of biopsies performed to confirm false-positive mammographic findings and more effectively to assess the results of treatment. With these improvements, there will be substantial economical savings and most importantly, a better overall patient care can be realized.

The fact that magnetic resonance techniques have become important in clinical imaging is well known. It is less appreciated the role of MRS as an effective technique in breast cancer research to study the metabolism of isolated intact cells and transplantable animal tumor models (15). Such studies can provide important information on biochemical processes and can be used for the identification of signals, as well as for the understanding of metabolic processes in vivo, i.e. the clinical application of MR spectroscopy. Perfused intact cells grown in culture conditions represent possibly the best approach to the non-invasive study of metabolism. In contrast to the in vivo situation, the cells are homogeneous, metabolically stable. The transplantable animal tumor model can provide information on tumor blood flow, metabolism and oxygenation. These are critical factors controlling the physiological environment within tumor and they are, directly and indirectly, major determinants of the outcome of nonsurgical forms of cancer therapy. The non-invasive nature of nuclear magnetic resonance (NMR) allows these parameters to be studied and understood better with minimal perturbation. The experimental manipulation of these parameters through different therapeutic methods can evolve into better strategies for treatment or even cure.

One of the major limitations of the application of NMR methods in vivo is low sensitivity. The sensitivity determines the ultimate cancer detection capability and the resolution in image and in spectrum. Much of the development in NMR instrumentation has been directed towards the improvement of sensitivity including using stronger magnet, better NMR probe design and new detection strategies. A major advantage of using higher magnetic field strength is the increased sensitivity brought about by the greater induced signal. However, it soon reaches its limits and becomes more difficult to achieve a higher field. An alternative approach to improve sensitivity is to reduce the noise associated with the detection. In this proposal, we will use a high temperature superconductor (HTS) working at very low temperature to reduce electronic noise and significantly improve the sensitivity of detection. It will dramatically increase the sensitivity and improves the resolutions in the ex vivo cell studies and in vivo animal studies. If it is in the patient care, it will shorten the MRI study time and more importantly improve the specificity of MRI and MRS studies.

In MR imaging or spectroscopy studies, the signal-to-noise ratio (SNR) determines the sensitivity of detection and limits the ultimate resolution. To improve SNR becomes extremely important for applications such as dynamic contrast enhancement study as well as the localized spectroscopy study in a small area. In order to improve SNR, much of the development in NMR instrumentation in the past has been to use larger sample volume and ever higher magnetic field to increase the NMR signal (22-25). It has proven to be very expensive and it has reached its limit. In contrast, an alternative approach is to reduce the noise associated with the detection, which will significantly improve SNR. Reducing the noise is the thrust of using a high temperature superconductor (HTS) as very low resistance coil material working at a very low temperature for both MRI and MRS studies.

There are two types of noise in an NMR study: the sample noise and the electronic noise. The sample noise generally can not be changed. However, the electronic noise including noises from the coil and from the preamplifier can be reduced. For a copper coil, the relative magnitude of the sample noise and the coil noise is dependent by the resonance frequency and the sample size. The NMR voltage has a w^2r^2 dependence, where w is the Larmor frequency and r is the linear dimension of the sample and receiver coil (27,28). The noise voltage of the sample is proportional to $wr^{3/2}$. The coil noise voltage is proportional to $w^{1/4}r^{1/2}$. The total SNR is a function of $w^2r^2/(w^2r^3+Aw^{1/2})^{-}$, where A is an empirical constant. Bulk, polycrystalline $Y_1Ba_2Cu_3O_7$ (YBCO) superconductor will be used to construct a small MRI and MRS probes. The overall SNR improvement can be realized depends on the relative importance of the noise from patient and the dimension of the coil. As the dimensions of the subject and RF coil are reduced, there is a point beyond which the RF coil contributes more to the noise than the patient. This point is dependent upon field strength, geometry and the quality factor of the coil with and without loading.(29). For a smaller coil used in NMR spectroscopy and microscopy, it is the thermal or that Johnson noise that dominates. The two parameters that determine the magnitude of Johnson noise, are resistance of the RF coil, and temperature. In this study, the HTS coil size will be one inch. The operation temperature will be the liquid helium temperature. A factor of four improvement of the SNR is expected.

III. METHODS

There are specific goals to be achieved for this four years project. The technical objective relevant to the first year are listed as following:

Probe Design: (1)To design and fabricate NMR probes using high temperature superconductor (HTS) material, $YBa_2Cu_3O_7$ thin film for a perfused-cell study on a 9.4 T NMR machine and for an in vivo animal study on a 4.7 T NMR machine. To design and construct the cryogenic systems which will include liquid nitrogen dewars, vacuum systems and connectors. To acquire cryogenic amplifiers which are specific for low-noise amplification at the designed frequency ranges. (2) To construct conventional copper probes of same size as the HTS probes for comparative studies.

Cell Metabolism Study: To obtain ^{31}P spectra of breast cancer cells MCF7, MCF7/III, MCF7/LCC2 and MCF7/LY2 in agarose gel not only to see the differences of characteristics of spectrum but also to document the improvement of HTS probe.

The methods relevant to achieve the goals in the first year are listed here.

Probe Design

Self-resonant probes for high resolution NMR studies will be fabricated from $YBa_2Cu_3O_{7-d}$ (YBCO) thin films. The films are 350-600 nm thick and they are grown on substrates including lanthanum aluminate, sapphire and magnesium oxide. The typical critical temperature T_c is about 90 °k in the absence of a magnetic field and the low frequency critical current density J_c is approximately 10^6 A/cm^2 at 77 °k. The HTS probes will be patterned by standard lithographic techniques. The coil dimension is comparable to the standard normal coils such that it excites a similar sample volume. For the perfused-cell studies at 9.4T (^{31}P at 162 MHz), the probe will be 10 mm long. the probe will fit in a 89 mm wide-bore magnet and the sample tube is 10 mm. The probes for in vivo animal studies at 4.7T (^{31}P at 81 MHz, ^{1}H 200 MHz) will also be 10 mm. The traces are integral interdigital capacitors are built in at the bottom for tuning to the desired resonance frequency. The resonance frequency f of a resonant circuit is determined by its inductance L and capacitance C, $2pf = (1/LC)$-. The height of the coil is selected to match the active sample length and the width is optimized for coupling. This geometry determines the inductance L of the coil. Interdigital capacitor C is used to tune the resonator to the desired frequency. The dielectric constants of samples will affect the resonance frequency. In order to compensate for the variation between samples, a metallic tuning paddle is added to adjust the resonance frequency through inductive coupling. The tuning range of such a design can be as large as a few MHz. The kinetic inductance of HTS varies with temperature so the resonance frequency of a HTS resonator also shows temperature dependence. In order to maintain the resonance frequency to within a narrow range of the bandwidth, the temperatures of the coil have to be stable. This stability will be achieved by a temperature controller and the coil temperature will also be regulated within a small range.

The HTS coil will be used both for receiving RF signals only. During transmission, a room temperature RF probe will be used. Low noise temperature of the coil place tighter requirements on the acceptable system noise figure. The system noise includes the noise from the amplifier and

the cable. The probes will be connected to the preamplifier by means of small weakly coupled loop. By adjusting the strength of the coupling, the probe impedance at resonance can be matched to the 50W noise impedance of a cryogenic low-noise amplifier (DOTY LN-2L or similar).

Studies of Cell Metabolism

We will study four different breast cancer cell lines including: MCF7, MCF7/MIII, MCF7/LCC2 and MCF7/LY2. These cell lines have been extensively studied and characterized, and exhibit specific phenotypic changes that reflect critical characteristics of the progressed phenotypes. Since MRS is a relatively insensitive method, it is necessary to have a large number of cells in each experiment and this somewhat limits its use to cell lines that can be grown in culture conditions. Perfused intact cells represents the best approach to the study of metabolism. All the substrates and nutrients can be continuously furnished and waste products removed, while stable pH levels and temperature are maintained. However, the perfusion cannot be performed with cells freely suspended in the NMR tube, since the flow would wash the cells away. The cells will be restrained by agarose threads.

The use of agarose threads was introduced by Dr. Cohen (co-investigator). It is a carbohydrate gel and is based on the properties of low-temperature gelling agarose (SeaPlaque), that allows mixing of cells with liquid agarose at 37°C, and solidification of the mixture at a lower temperature. 1-1.2 ml of cell pellet ($2\pm0.5 \times 10^8$ cells) are mixed with equal volume of 1.8% liquid agarose in phosphate-buffered saline, and immersed in a bath at 37°C for 5-7 min. The mixture is extruded under low pressure through cooled tubing (0.5 mm id) into a 10 mm MRS tube containing growth medium. Using 0.5 mm threads ensures that there is no metabolic compromise, and the cells are viable and in stable energetic status for more than 24 h, while the threads maintain their mechanical strength. The gel threads which fill the tube are concentrated without compression at the bottom of the tube by insertion of a plastic insert with the perfusion fittings. The inflow tube, made of Teflon, is 0.5 mm id, and is placed near the bottom of the tube. The outflow is directed into openings in the insert, and then into an outflow tube. Perfusion rates (0.3-2 ml/min) are maintained by a peristaltic pump, and since the Teflon tubes are permeable to air, it is not required to include a gas exchanger in the perfusion system. Perfusate can be recycled or can be wasted, and the cells are continuously perfused with fresh medium. A debubbler is inserted into the apparatus prior to the insert to remove air bubbles, which may spoil magnetic field homogeneity and affect perfusate flow. The perfusion solution will be the buffered growth medium that is most appropriate for the cells studied.

IV. RESULTS AND DISCUSSIONS

1. Relocation of the 400 MHz machine and preparation of the studies

The 400 MHz NMR machine has been relocated from Georgetown University to the Biomedical NMR Laboratory at Howard University. The 400 MHz machine is the system designated for the cell metabolism study. Moving the 400 MHz machine to the same laboratory with the 200 MHz machine and other instruments improves the logistics of the research. The moving and recharging of the magnet and setup of the NMR console have completed. The

system is up and running. The functions of temperature control and shimming of the 400 MHz machine are normal. A phosphoric acid in a 10 mm tube with the same volume as in the proposed perfusion cell study is used to check the shim. A P^{31} spectrum of phosphoric acid is shown in Figure 1. The line width of the spectrum is 2.1 Hz. A standard sample, 10% TMP in C_6D_6, was used to check the resolution and signal-to-noise (S/N) ratio. For the room temperature probe, the S/N is about 400 (Figure 2). The line width of the central peaks is 1.02 Hz.

As part of the support, the university has renovated two laboratories for this project. One of the laboratory accommodates both the 360 MHz and 400 MHz machine. The other laboratory is for the chemical and biological preparation. A large amount of laboratory supplies and the cell culture materials have been ordered. A computer and an imaging processing software, Analyze, have been purchased. With the new computer and the software, the lab is ready for the off-line image and spectra processing. Other new equipment provided by the university have been ordered and partially received including a refrigerator and a de-ionizing water column.

2 Design and Construct: HTS probes and room temperature probes

One of the major tasks in the initial 18 months of the project is to design and construct the HTS probes with Quantum Magnetics Company, San Diego, CA. These HTS probes will be used in P^{31} NMR studies of breast cancer cells in the culture media and breast cancer tumors in animals over the next two and half years. The HTS probes consist of a superconductor receiver coil, a preamplifier and a cryogenic cooling system. The HTS probes will be made in such a way that the superconductor coils are detachable from the assembly. The same preamplifier and the cryogenic system are used for both 200 MHz and 400 MHz machines. Under this scheme, only the HTS coil needs to be switched when using different probes in different magnets. The Quantum Magnetics with IBM will construct the superconductor receiver coils and procure the preamplifier and the cooling system. Several revisions have been made from the original design. The probe temperature will be of liquid helium temperature instead of liquid nitrogen temperature. This revision is due to the fact that the high temperature superconductor, YBCO, performs with more stability at lower temperatures. The RF transmission will be done by a room temperature RF coil perpendicular to the HTS coil. This design will avoid the potential cross talk between the transmission and receiving coils. It is necessary to separate the transmission and receiving functions because the HTS coils sustain only a limited current, which may not be sufficient for the proposed studies. The receiver coil, preamplifier and the cryostat will reside in the lower half of the magnet while the sample and the room temperature transmitter will be placed from the top of the magnet. A detailed design of the HTS probes are shown in Figure 3 and 4. In order to verify the S/N improvement of the HTS probes, similar room temperature probes are needed. A 3-turns loop P^{31} coil made of copper wire has been constructed to be used for the 200 MHz machine. The construction of the room temperature P^{31} coil for the 400 MHz machine is in progress.

The cryostat to be used is Spectrostat from Oxford Instrument (Fremont, CA). It is constructed from non-magnetic materials and components for use in NMR measurements. It has optimal thermal design providing excellent control and stability of the HTS coil and the sample temperatures. The outer diameter of the Spectrostat will perfectly fit in the 400 MHz machine, 89

mm magnet bore. The cryostat comprises of a heat exchanger, sample space, radiation shield and vacuum case. It provides a continuous flow of liquid helium. Liquid helium flows from a storage vessel and is drawn through a flexible, vacuum-insulated transfer tube and circulated through the dewar containing the HTS coil. The preamplifier to be used is LN-2H from Doty Scientific, (Columbia, SC). The preamplifier is intended for lowest possible noise and fastest possible recovery from saturation. The noise figure is below 1 dB at 100 MHz.

3. Construction and testing of the cell perfusion apparatus

Three identical cell perfusion apparatus for the NMR study of breast cancer cell metabolism have been constructed. The apparatus consists of a 10 mm glass NMR tube, a custom-made screw-on cap, an inlet and an outlet opening for the Teflon tubing, and fitted O-rings for air-tight seals. The detailed design of the perfusion apparatus is illustrated in Figure 5. The screw-on cap is made of Kel-f material. Kel-f has good thermal properties as well as very low magnetic susceptibility. These properties are critical to ensure minimal effects of the cell perfusion apparatus material on the observed NMR signal. After the construction, the cell perfusion apparatus was tested on the 400 MHz machine to check its functions. A peristaltic pump was connected to the inlet of the tubing and the flow rate was adjusted to simulate the conditions of the in vitro experiment. Silicon oil was used to seal the cap openings of the apparatus.

4. Cell metabolism study

To study the metabolism of breast cancer cells in an extended period of time, the cells need continuous perfusion of nutrients. During perfusion, the breast cancer cells are restrained in the agarose gel-thread matrices. Making agarose gel-thread matrices requires practice. We have successfully made agarose gel-thread matrices containing MCF7 wild type breast cancer cells using the following protocol. A solution of low gelling temperature agarose (SeaPlaque@ agarose, FMC) was dissolved in saline (0.9% NaCl). The agarose concentration is 1.8% w/v. The solution was heated to near boiling to completely dissolve the agarose. The solution is then stored in the liquid state at 37^0C water bath ready for use. The MCF7 wild type breast cancer cells in the growth medium are mixed with the agarose solution in a 1:1 ratio. Total of 2 ml of the mixture is used for each perfusion experiment. The gel-thread making setup is shown in Figure 6. The gel matrix is forced to extrude under mild pressure through a coil of Teflon tubing (i.d. 0.5mm) into a NMR tube containing cell growth medium. The pressure is provided by a peristaltic pump through another Teflon tubing into the sealed plastic tube containing the mixture. The coil of Teflon tubing is about 42 inches. It is chilled in an ice bath. The length of Teflon tubing is important because it needs to be long enough to ensure the gel matrix becomes gel when it extrudes into the icy medium bath. However, the length of the tubing can not be too long to coagulate the tubing. Three or four turns of the coil are wrapped around a small cylinder and immersed in the ice water mixture to ensure the quick gelling process of the cell-gel mixture.

The agarose gel-thread matrix in a NMR tube is then gently pushed to the bottom by inserting the perfusion apparatus. The setup of the perfusion is shown in Figure 7. Using a room temperature probe, a P^{31} spectrum of the perfused agarose gel-thread matrices containing MCF7

cancer cells is obtained in Figure 8. The spectrum is a summation of 16000 scans with the NMR repetition time 1.3 sec. The total scan time is 6 hours. The peaks on the spectrum associated with phosphoethanolamine (PE), phosphocholine (PC), inorganic phosphate (Pi), glycerophospho-ethanolamine (GPE), glycerophosphocholine (GPC), adenosine triphosphate (ATP) and diphosphodiester (DPDE) can be clearly identified. From the accumulated spectra over eight hours, it demonstrated the breast cancer cells are viable during the experiment time. The perfusion setup is working properly.

5. Upgrade of the 200 MHz NMR machine and implementation of a diffusion imaging technique

The software for the 200 MHz NMR machine has been upgraded. The upgrade includes two parts: the workstation operating system upgrade and the NMR software upgrade. The software from Varian has been upgraded to VNMR 5.3 B with an option to VNMR 6.0 when it is available. The new software allows the oblique imaging with an arbitrary angle. It also has many advanced imaging processing routines in the image browser. The new image browser enables us to perform sophisticated image processing functions, which are important for the micro-imaging of the tumor vasculature. The operating system on the Sun SPARC workstation has been upgraded to Solaris 2.5.1.

The study of the permeability or leakiness of the capillaries in tumors will be an important part of the in vivo study. Diffusion weighted imaging technique has been implemented. Although the implementation of the diffusion imaging technique is not one of the objectives in the first year, however, it will be used in the imaging of tumor in later years. It will provide the information of drug delivery within the tumors. Since the construction of the HTS probes has been delayed, we decided to use the time to start preparing the in vivo animal studies by implementing the necessary imaging techniques. A test phantom consisting of four bottles containing water, ethanol, acetone and copper-sulfate solution is used to test the diffusion imaging sequence. A diffusion map of the phantom was calculated from a series of diffusion weighted images with different magnetic field gradients and gradient on time. The strongest magnetic field gradient on this machine is 60 Gauss/cm. Diffusion coefficients of the solutions obtained are in consistent with the values in the literature.

V. CONCULSION

In the first year of the project, construction of HTS probes is slightly behind schedule. Part of the reason is due to refining the probe design. The setup for cell perfusion has been completed. In the mean time, the cell metabolism study and the preparation of the in vivo animal study have begun.

The HTS probe design and construction are well underway. It is expected to be completed in the next six months. The probes have been constructed at Quantum Magnetics Company. The HTS coils have been fabricated. The preamplifier and cryostat have been procured by Quantum Magnetics. Three cell perfusion apparatus have been made and tested. They have been used to check the basic functions of the 400 MHz machine. We have started to

grow the wild type MCF7 cells in culture medium. We have also successfully grown MCF7 cells in agarose gel for the cell metabolism study. A six-hours long P^{31} study of the perfused MCF7 cells in agarose gel has demonstrated the integrity of the perfusion setup. The 400 MHz machine has moved to the new laboratory next to the other NMR machines. This improves the logistics of the research. Two new laboratories have been renovated to accommodate the NMR machines and cell biology preparation work. Much equipment and computer hardware and software have been ordered and delivered. Major software upgrade has been done on the 200 MHz machine. A diffusion imaging technique has been implemented on the 200 MHz machine. It is ready for the in vivo micro-imaging study of tumor vasculature and drug delivery inside the tumor.

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VII. APPENDICES

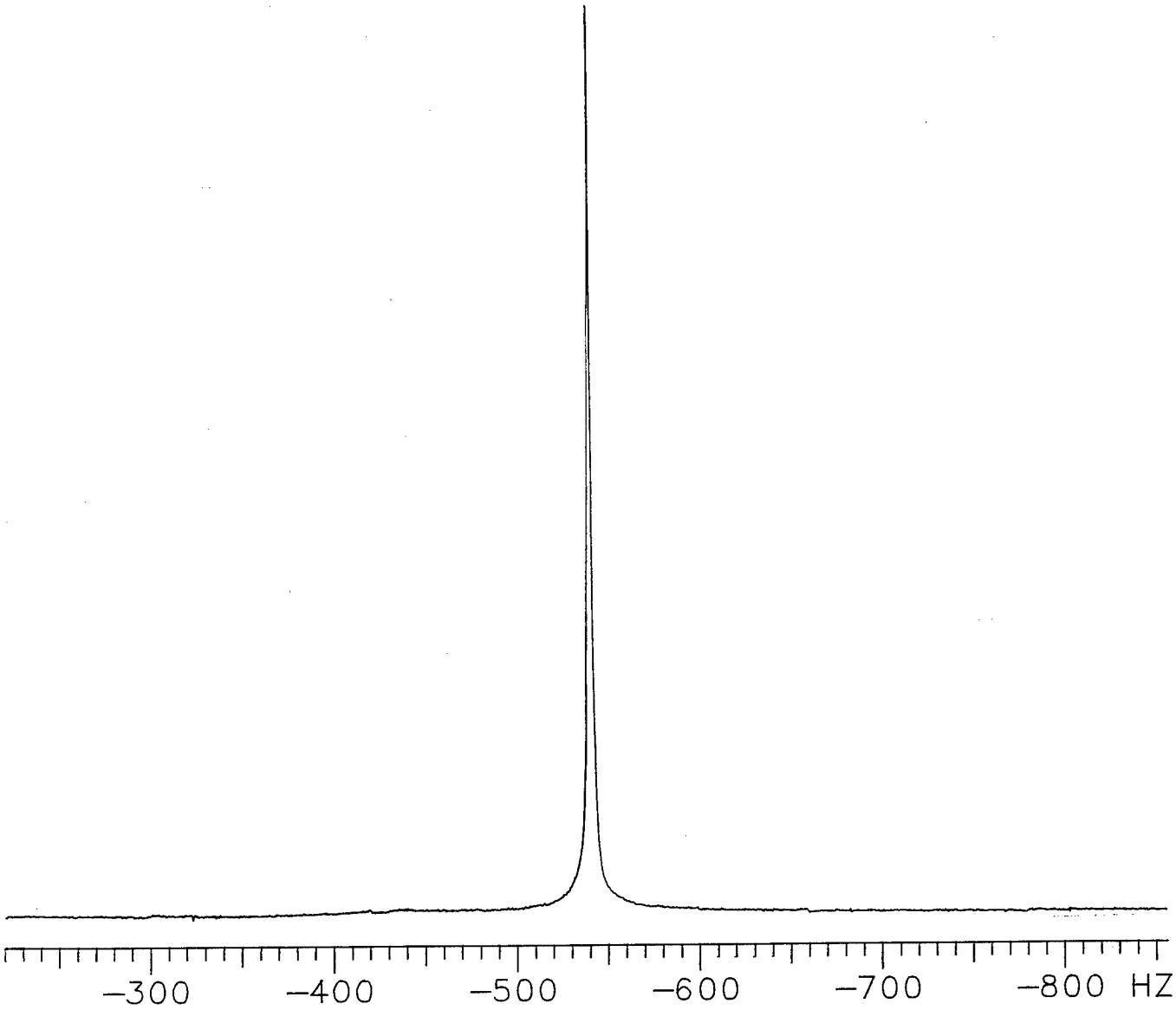

Fig. 1 A ^{31}P NMR spectrum of phosphoric acid. The spectrum is a single scan taken from phosphoric acid solution of same volume as in cell perfusion studies. The line width of the spectrum is 2.11±0.21 Hz. With a line broadening of 1Hz, the S/N is measured to be 700. The sweep width is 4000 Hz and 16384 data points were taken for an acquisition time of 2 seconds.

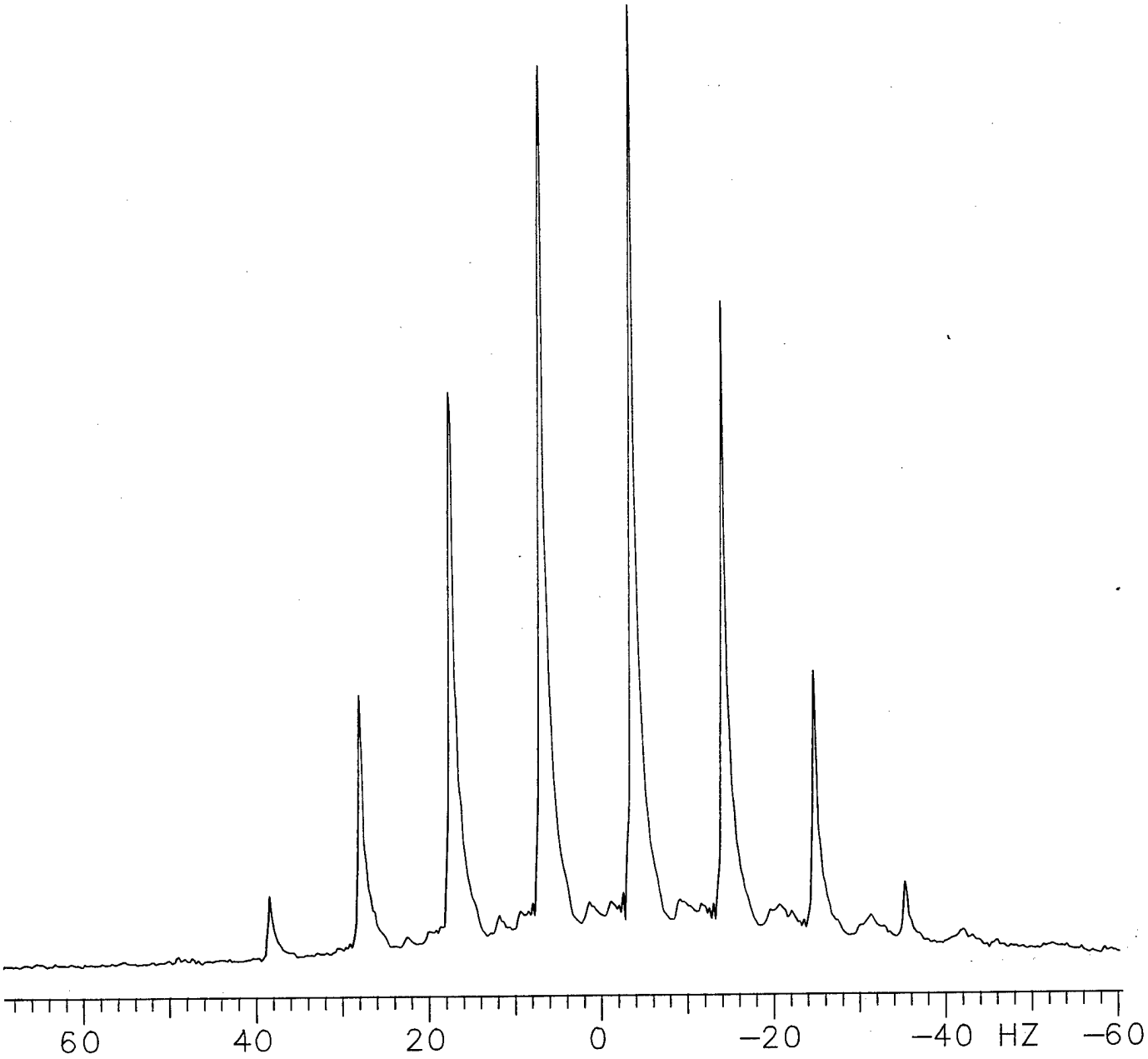

Fig. 2 A ^{31}P NMR spectrum of 10% trimethyl phosphine (TMP) in C_6D_6 at room temperature, using 400 MHz spectrometer XL-400. The number of scan is one. The line width of the central peaks of the spectrum is 1.05 ±0.18 Hz. With a line broadening of 1Hz, the S/N is 623. The sweep width is 4000 Hz and 16384 data points were taken during the acquisition time of 2 seconds. The sample spinning rate was 15 s^{-1}.

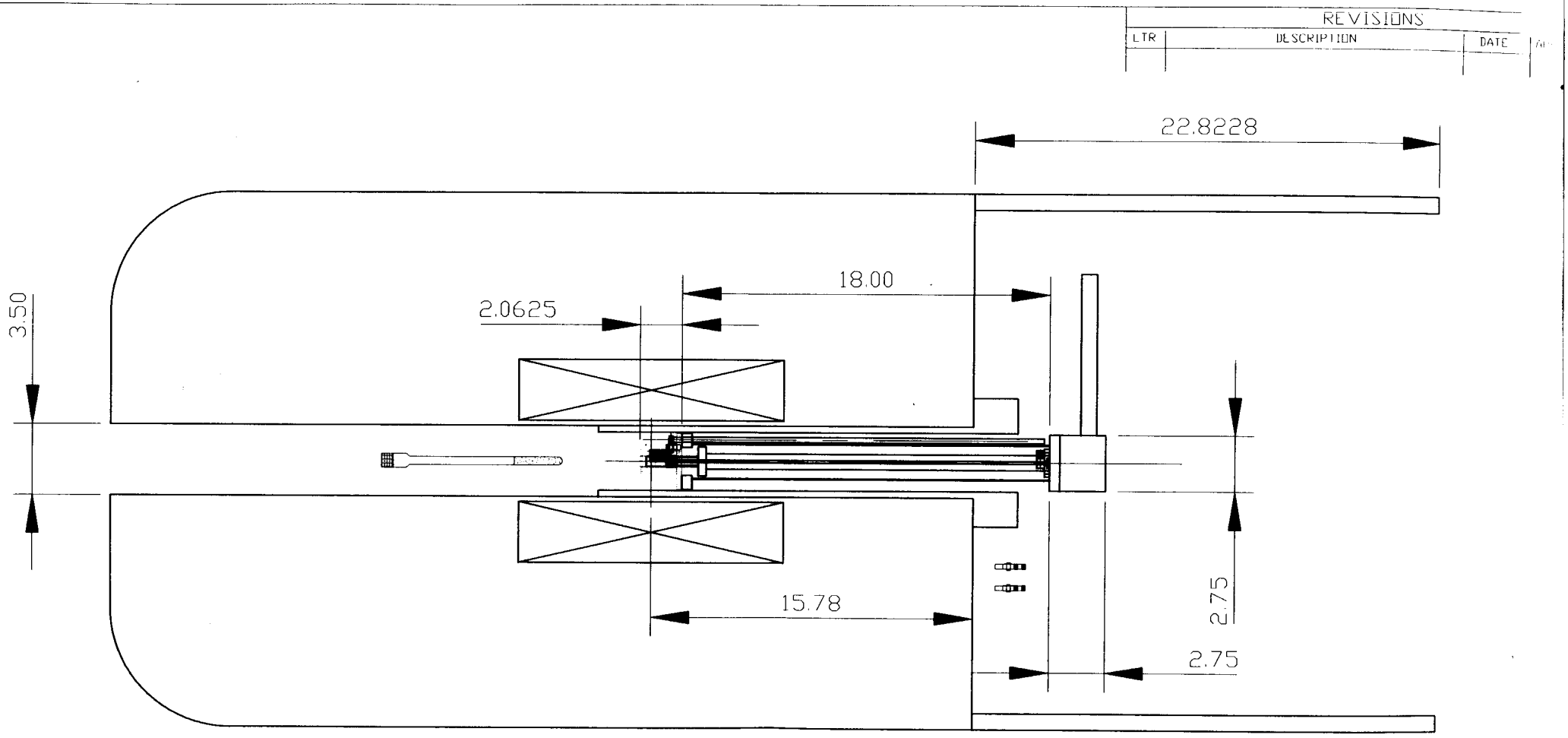

Fig. 3 A side view of the high temperature superconductor probe in the XL-400. The HTS probe is loaded from the bottom of the magnet.

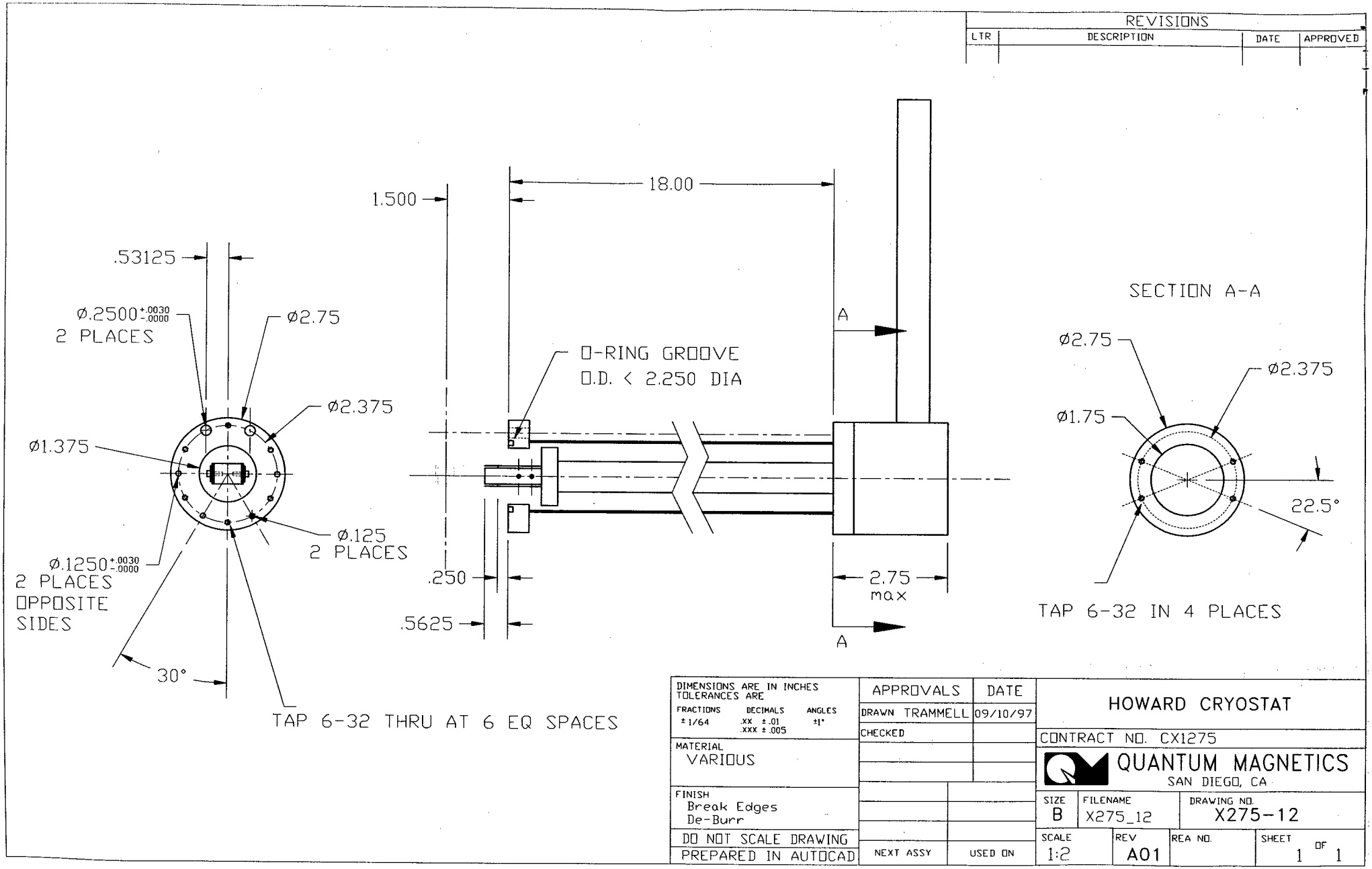

Fig. 4 A detailed drawing (side view and top view) of the cryostat of the HTS probe. This is the insert section, which goes into the magnet from the bottom as shown in Fig. 3.

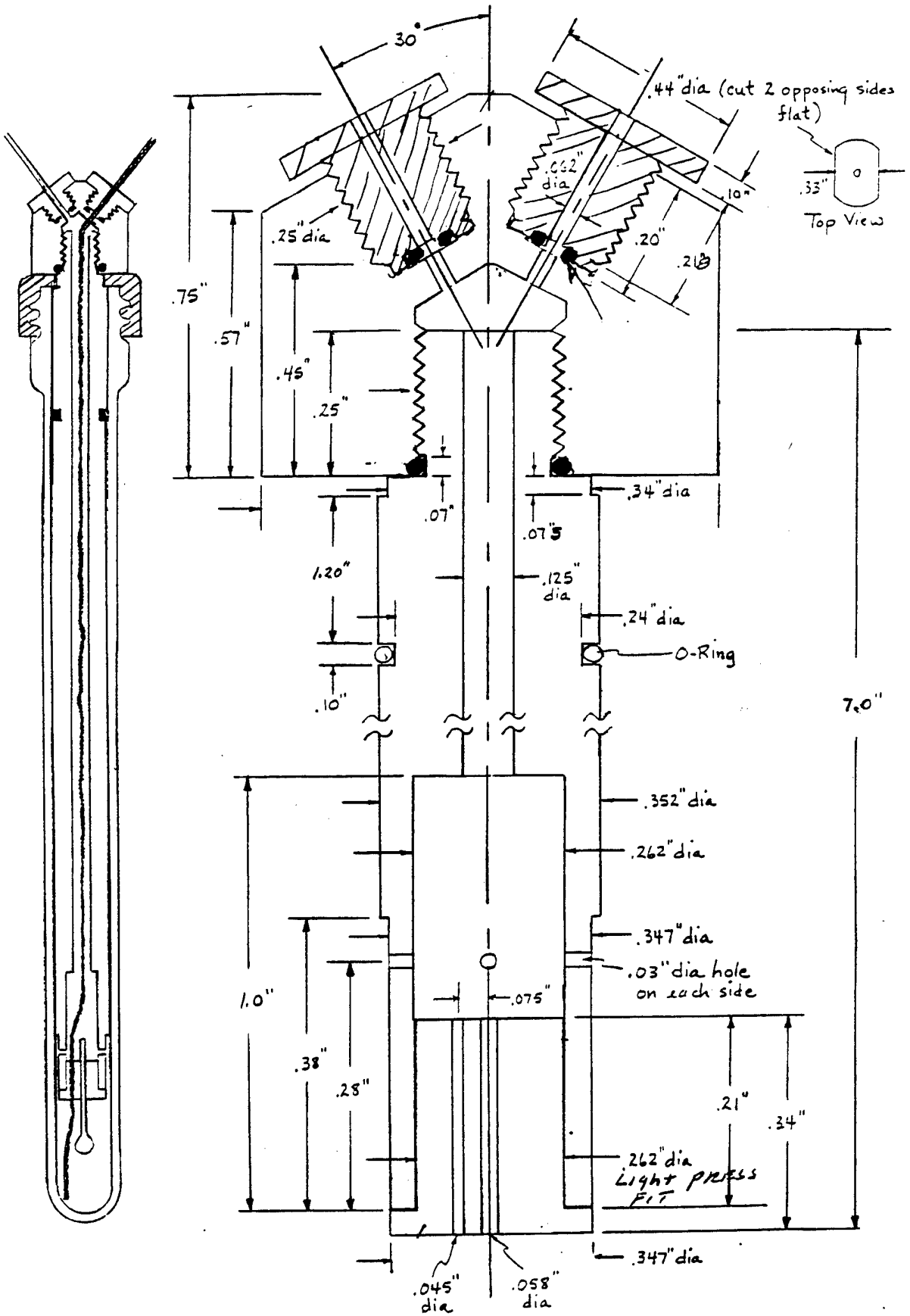

Fig. 5 A detailed design for the cell perfusion apparatus. A schematic drawing of the apparatus in a NMR test tube is shown on the left.

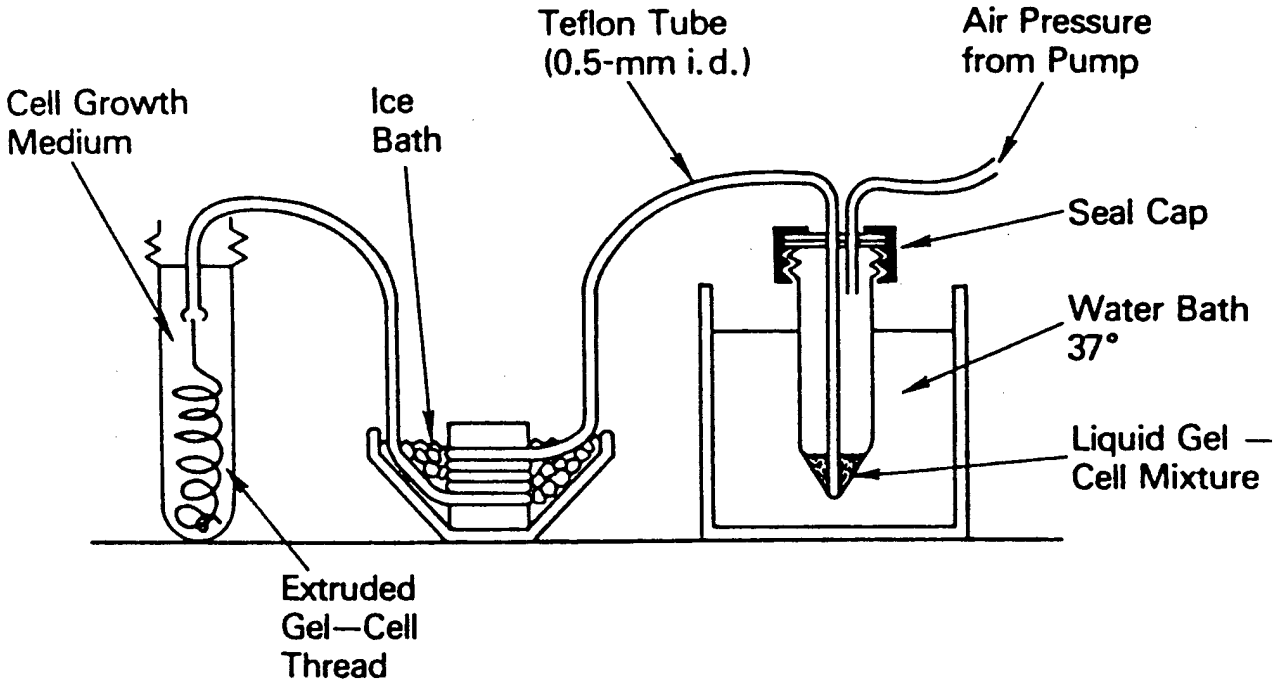

Fig. 6 A diagram of the apparatus for making agarose gel-thread containing breast cancer cells. A mixture of cancer cells with agarose solution is extruded through a fine Teflon capillary tubing chilled by ice. The gel-thread is formed in the tubing and it is forced out directly into the medium in a 10-mm NMR tube ready for perfusion.

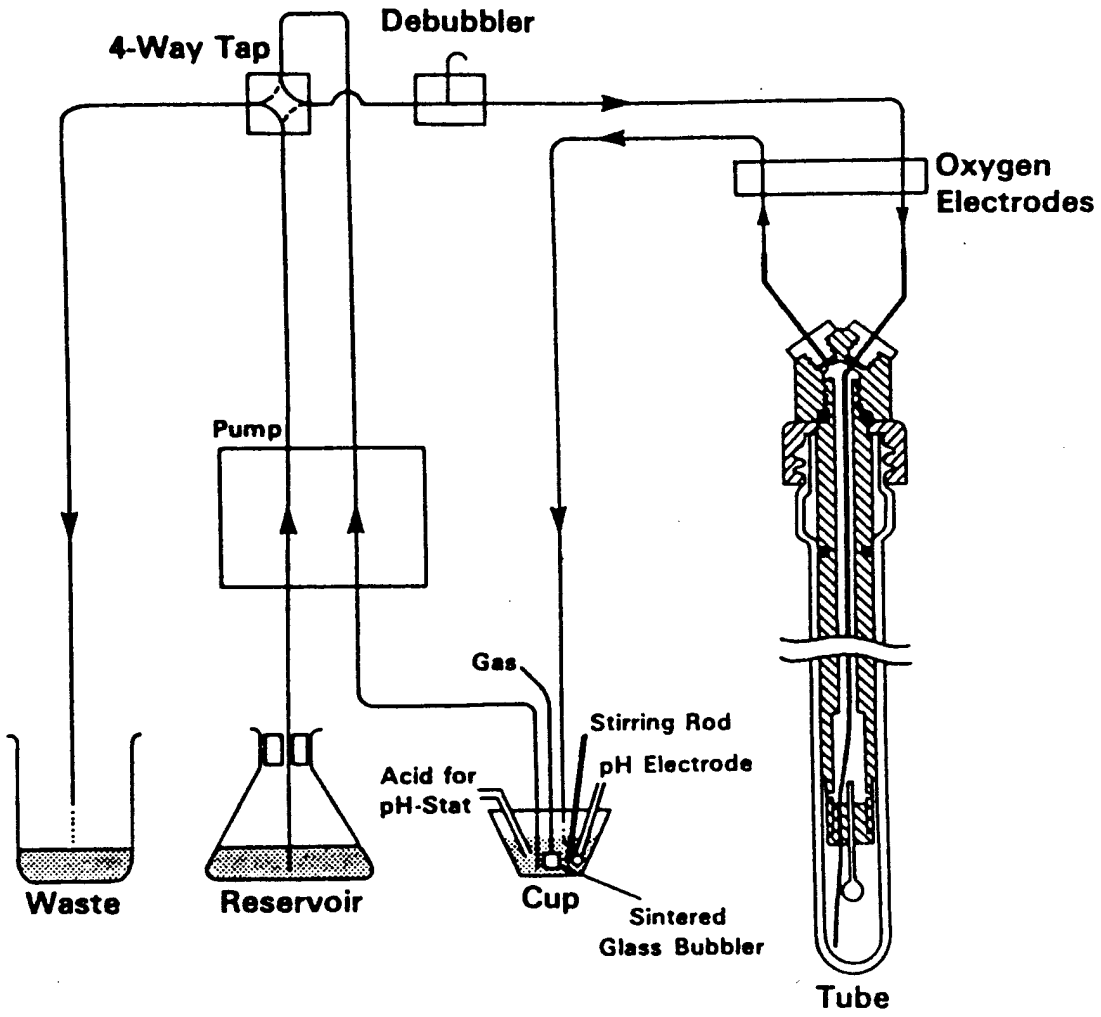

Fig. 7 A schematic diagram of the perfusion system. The figure shows the Kel-F plastic insert in a NMR tube and the flow direction of the perfusion medium.

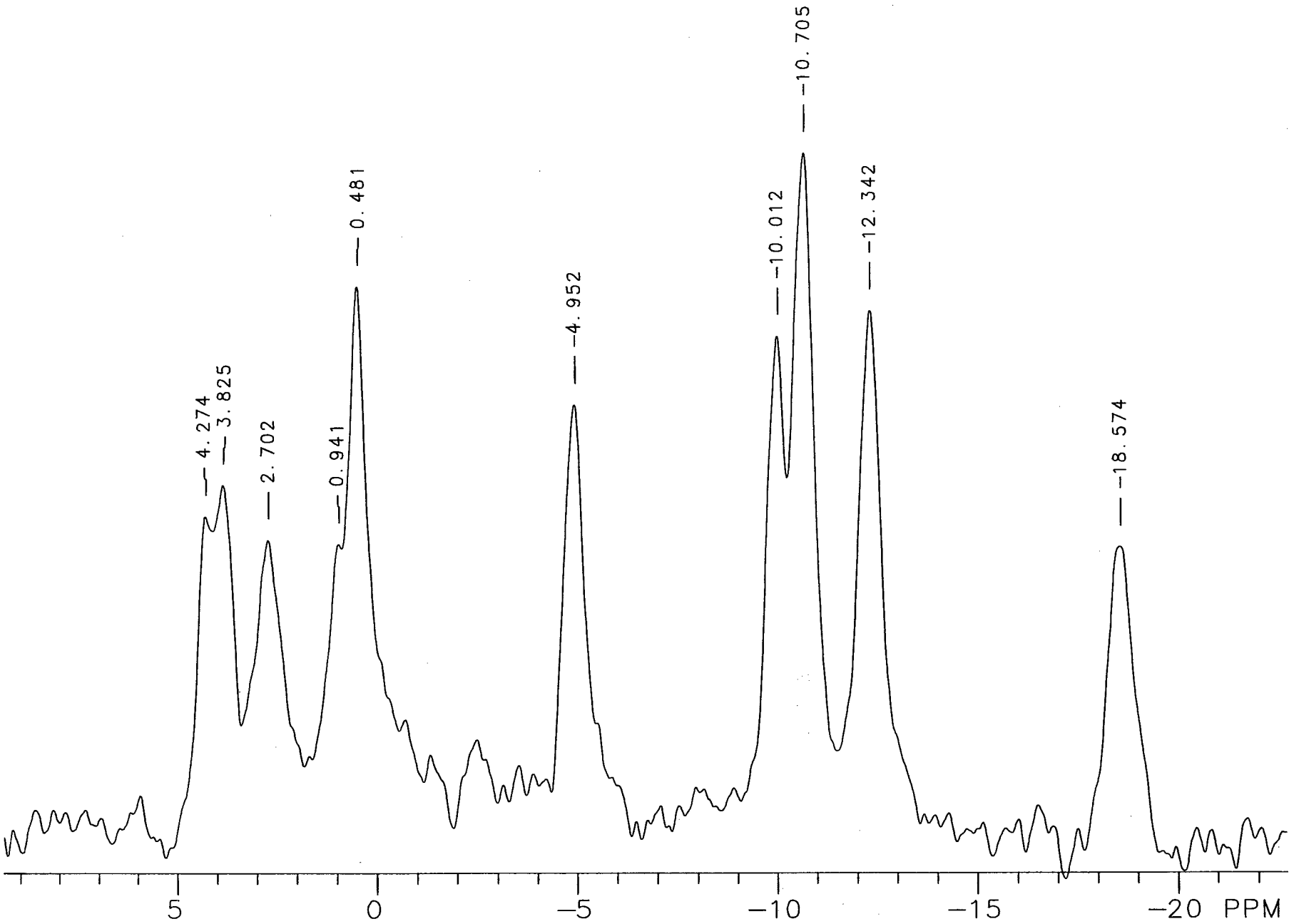

Fig. 8 A ^{31}P NMR spectrum of perfused MCF-7 breast cancer cells. The spectrum is a sum of 8 spectra. Each one has 2000 scans with a repetition time of 1.3 seconds. The total time for getting this spectrum is about 6 hours. The S/N of this spectrum is 34. The peak assignments are: 4.27 ppm, phosphoethanolamine (PE); 3.83 ppm, phosphocholine (PC); 2.70 ppm, P_i; 0.94 ppm, glycerophosphoethanolamine (GPE); 0.48 ppm, glycerophosphocholine (GPC); -4.95 ppm, γ-ATP; -10.01 ppm, α-ATP; -10.71 plus -12.34 ppm, diphosphodiesters (DPDE); -18.57 ppm, β-ATP.